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Note

Steroid 5α-Reductase Inhibitory Activity and Hair Regrowth Effects of an Extract from *Boehmeria nipononivea*

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The acetone extract of *Boehmeria nipononivea* showed both potent 5α-reductase inhibitory activity and hair regrowth promotion effects on mice. 5α-Reductase inhibitory activity-guided fractionation led to six active fatty acids: α-linolenic, linoleic, palmitic, elaidic, oleic and stearic acids. The extract of *B. nipononivea*, and α-linolenic, elaidic and stearic acids exhibited a hair regrowth effect.

Key words: *Boehmeria nipononivea*; hair regrowth; testosterone 5α-reductase inhibitor; fatty acid; Urticaceae

The role of androgens in the development of male baldness is clear, but the mechanism is still obscure. There are some reports that bald scalp skin in men had an elevated 5α-reductase (Δ^4-3-oxo-steroid 5α-oxidoreductase: EC 1.3.99.5) level,[1,2] and that men with a genetic 5α-reductase deficiency did not develop androgenetic alopecia.[3] During the last few years, a class of specific and potent 5α-reductase inhibitors has been synthesized,[4] and one compound, finasteride, has been developed and marketed for treating benign prostatic hyperplasia. This drug has been shown to lower the serum and intraprostatic dihydrotestosterone levels in men.[5] Studies on finasteride have shown that the inhibition of 5α-reductase can reverse the balding seen with age in both the male and female stumptail macaque (*Macaca arctoides*).[6] These observations have led to the hypothesis that 5α-reductase inhibitors might be useful for treating androgenetic alopecia such as male baldness.

The purpose of this investigation is to identify extracts or components from plants having both a hair regrowth promotion effect and 5α-reductase inhibitory activity.

5α-reductase inhibitory activity was assayed by the previously reported method.[7] The hair regrowth effect was assayed by using male mice of the C57/Black strain at 5 weeks old. Six cm^2 (3 × 2 cm) of dorsal hair was shaved with an electric shaver and removed by using the depilatory agent, EBA cream (Tokyo Tanabe Co. Ltd., Japan), to avoid injury to the skin. To the back was applied 200 μl of a 2% sample in EtOH as a vehicle once a day for 20 days. The index of the area of the hair regrowth to the shaved area was individually calculated as the brightness (Y%) by a CR-100 colorimeter (Minolta Co. Ltd., Japan) and compared in each group of mice. The brightness data were analyzed by Student's t-test, differences of $P < 0.05$ being regarded as significant. Experiment 1 was conducted on the acetone extract of *B. nipononivea*, elaidic acid, α-linolenic acid and linoleic acid, while experiment 2 was conducted on oleic acid, stearic acid and palmitic acid. It should be noted that we used the C57/Black mouse, which has a highly synchronized hair cycle that can be easily evaluated in an assay of hair regrowth, but is not an animal model for androgen-dependant baldness such as the stumptail macaque. We therefore cannot propose any direct relationship between the hair regrowth effect and the 5α-reductase inhibitory activity.

The leaves of 10 plant species (*Achyranthes bidentata, Boehmeria nipononivea, Artemisia* sp., *Boehmeria longispica, Boehmeria platanifolia, Boehmeria sieboldiana, Pellionia scabra, Pellionia minima, Aucuba japonica, Dioscorea japonica*) were obtained in Fukuoka City (Japan). The chopped aerial of each (5 g) was extracted with acetone (100 ml) for 7 days at room temperature, and the resulting extract was concentrated to dryness. We investigated the 5α-reductase inhibitory activity of these acetone extracts, the results being shown in Table 1. In this investigation, the extracts from all the *Boehmeria* species examined showed high inhibitory activity. Since *B. nipononivea* was the most readily available of these *Boehmeria* species for us, this species was selected for further investigation. The acetone extract of *B. nipononivea* was then partitioned between *n*-hexane and diethyl ether. The 5α-reductase inhibitory activity of the *n*-hexane-soluble, diethyl ether-soluble and

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Table 1. 5α-Reductase Inhibitory Activity of Acetone Extracts (Sample concentration: 480 μg/ml)

Sample	Inhibition (%)
Boehmeria nipononivea	70.0
Boehmeria platanifolia	89.1
Boehmeria sieboldiana	81.5
Boehmeria longispica	77.2
Dioscorea japonica	65.0
Pellionia minima	40.9
Pellionia scabra	30.8
Aucuba japonica	23.0
Achyranthes bidentata	83.5
Artemisia sp.	79.4

aqueous portion was 81.7%, 58.9% and 28.2% at a concentration of 62.5 μg/ml, respectively. The *n*-hexane-soluble portions showing the strongest 5α-reductase inhibitory activity were submitted to further activity-guided separation by silica gel CC and preparative HPLC, giving several active fractions. GC-MS and LC-MS analyses identified the compounds in the active fractions as α-linolenic acid, linoleic acid, palmitic acid, oleic acid, elaidic acid and stearic acid. The 5α-reductase inhibitory effect of the acetone extract from *B. nipononivea* thus appeared to be attributable to fatty acids. Therefore, the 5α-reductase inhibitory activity of several of the fatty acids identified in the active fractions from the acetone extract of *B. nipononivea* was examined the activity at 31.3 μg/ml and 62.5 μg/ml, respectively, being as follows: α-linolenic acid (88.0%, 95.9%), linoleic acid (70.7%, 97.4%), oleic acid (50.6%, 95.2%), elaidic acid (35.5%, 60.6%), stearic acid (32.4%, 45.3%), and palmitic acid (23.6%, 31.2%). The 5α-reductase inhibitory effects of these fatty acids were similar to the reported data.[8] The fatty acid composition in each acetone extract of the 10 plant species was then examined by GC-MS after a treatment with CH_2N_2. As can be seen in Fig. 1, the main fatty acids were α-linolenic acid, linoleic acid and palmitic acid in each acetone extract, other fatty acids being in trace amounts. The acetone extracts which showed potent 5α-reductase inhibitory activity

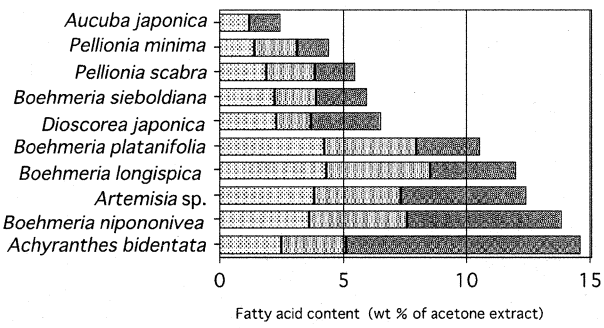

Fig. 1. Fatty Acid Composition of the Acetone Extracts from 10 Plant Species.
Palmitic acid Linoleic acid α-Linolenic acid

of more than 70% all had fatty acid contents greater than 10% (Table 1 and Fig. 1). These results indicate that some specific herbaceous plants are valuable as natural resources containing fatty acids having a 5α-reductase inhibitory effect.

The acetone extract of *B. nipononivea* and the main fatty acids were also investigated for their hair regrowth effect on mice. Brightness plots are shown in Figs. 2 and 3. As can be seen in Fig. 2, the acetone extract of *B. nipononivea* showed a significant hair regrowth promotion effect between days 15 and 22. Visible hair regrowth and the decrease in brightness were mutually correlated (data not shown). Fig. 2 shows the brightness plots after the application of α-

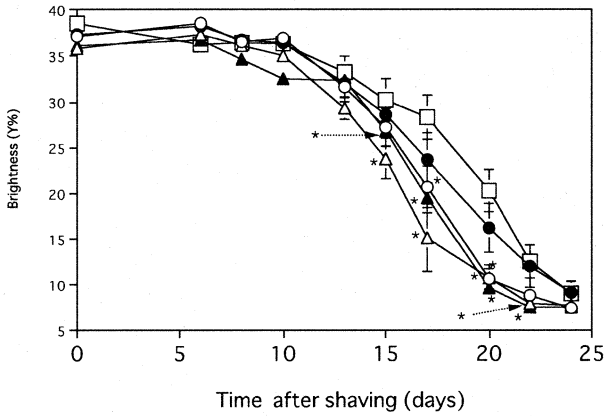

Fig. 2. Effects of the Acetone Extract of *B. nipononivea*, and of α-Linolenic Acid, Linoleic Acid and Elaidic Acid on the Hair Growth of Mice (Experiment 1)
Each value represents the mean ± SEM. *$p<0.05$ as compared with the control, n = 9
α-Linolenic acid Acetone extract
Linoleic acid Control (EtOH)
Elaidic acid

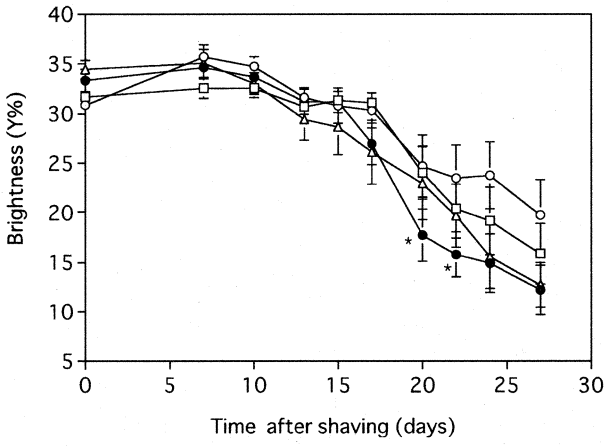

Fig. 3. Effects of Oleic Acid, Stearic Acid and Palmitic Acid on the Hair Growth of Mice (Experiment 2).
Each value represents the mean ± SEM. *$p<0.05$ as compared with the control, n = 9
Control (EtOH) Oleic acid
Stearic acid Palmitic acid

linolenic acid, linoleic acid and elaidic acid. α-Linolenic acid and elaidic acid showed significant hair regrowth promotion effects between days 17 and 20, and between days 15 and 22, respectively. Fig. 3 shows the brightness plots after the application of oleic acid, stearic acid and palmitic acid. Stearic acid showed a significant hair regrowth promotion effect on the 20th and 22nd days.

The potential of 5α-reductase inhibitors for developing therapeutic materials for androgen-dependent diseases such as benign prostatic hyperplasia, prostatic cancer, acne, seborrhea, common baldness, hirsutism, and hidradenitis is evident, if safety can be guaranteed. In this present work, it was found that acetone extracts of several herbaceous plants showed potent 5α-reductase inhibitory activity, the active constituents being several fatty acids (α-linolenic, linoleic, palmitic, elaidic, oleic and stearic acids). Therefore, some specific herbaceous plants would be valuable as natural resources containing fatty acids which have a 5α-reductase inhibitory effect.

It seemed that the acetone extract of *B. nipononinvea* and three fatty acids (α-linolenic, elaidic and stearic acids) possessed hair regrowth effects. The reason for the hair growth effect of fatty acids is not clear. Taking into consideration that these fatty acids were identified as 5α-reductase inhibitors only by 5α-reductase inhibitory activity-guided fractionation, there is a possibility that constituents in the acetone extract of *B. nipononinvea*, other than 5α-reductase inhibitory compounds contributed to the hair regrowth effect on mice.

Compounds which have both a hair growth promotion effect and 5α-reductase inhibitory activity could be candidates for useful hair growth stimulating preparations. The results of the present study indicate that the extract of *B. nipononinvea* and three fatty acids (α-linolenic acid, elaidic acid and stearic acid) had both 5α-reductase inhibitory activity and a hair regrowth stimulation ffects.

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References

1) Bingham, K. D., and Shaw, D. A., The metabolism of testosterone by human male scalp skin. *J. Endocrinol.*, **59**, 111–121 (1973).
2) Schweikert, H. U., and Wilson, J. D., Regulation of human hair growth by steroid hormones. I. Testosterone metabolism in isolated hairs. *J. Clin. Endocrinol. Metab.*, **38**, 811–819 (1974).
3) Imperato-McGinley, J., Guerroro, L., Gautier, T., and Peterson, R. E., Steroid 5α-reductase deficiency in man: an inherited form of male pseudohermaphroditism. *Science*, **186**, 1212–1215 (1974).
4) Rasmusson, G. H., Reynolds, G. F., Steinberg, N. G., Walton, E., Patel, G. F., Liang, T., Cascieri, M. A., Cheung, A. H., Brooks, J. R., and Berman, C., Azasteroids: structure-activity relationships for inhibition of 5α-reductase and of androgen receptor binding. *J. Med. Chem.*, **29**, 2298–2315 (1986).
5) McConnell, J. D., Wilson, J. D., George, F. W., Geller, J., Pappas, F., and Stoner, E., Finasteride, an inhibitor of 5α-reductase, suppresses prostatic dihydrotestosterone in men with benign prostatic hyperplasia. *J. Clin. Endocrinol. Metab.*, **74**, 505–508 (1992).
6) Rhodes, L., Harper, J., Uno, H., Gaito, G., Audette-arruda, J., Kurata, S., Berman, C., Primka, R., and Pikounis, B., The effects of finasteride (Proscar) on hair growth, hair cycle stage, and serum testosterone and dihydrotestosterone in adult male and female stumptail macaques (Macaca arctoides). *J. Clin. Endocrinol. Metab.*, **79**, 991–996 (1994).
7) Shimizu, K., Fukuda, M., Kondo, R., and Sakai, K., The 5α-reductase inhibitory components from heartwood of *Artocarpus incisus*: Structure-activity investigations. *Planta Med.*, **66**, 16–19 (2000).
8) Liang, T., and Liao, S., Inhibition of steroid 5α-reductase by specific aliphatic unsaturated fatty acids. *Biochem. J.*, **285**, 557–562 (1992).